Correspondence

AIDS 2024, **38**:1601–1605

Primary HIV infection during chronic treatment with imatinib: impact on infection dynamics

International guidelines advocate early combined antiretroviral treatment (cART) initiation to reduce the HIV-1 viral load set-point and diminish the reservoir size. Tyrosine kinase inhibitors (TKIs), used for blood malignancies and able to interfere with both T-cell activation and homeostatic proliferation, might have a role in suppressing viral replication, modulating the immune response, and limiting reservoir seeding during early HIV infection [1,2]. Ex vivo, TKIs dasatinib and ponatinib have been shown to maintain the antiviral activity of SAMHD1, a phosphohydrolase that inhibits HIV reverse transcription by depleting intracellular nucleotides pools [3–6]. Specifically, TKIs inhibit SAMHD1 phosphorylation, a posttranslational modification that blocks its antiviral activity [7–10].

Herein, we report the case of a man acquiring primary HIV infection at 37 years of age, whereas already undergoing treatment with TKI imatinib for a myeloproliferative neoplasm. Through the GR-2018-12365699 study, enrolling antiretroviral therapy (ART)-naïve participants with primary HIV infection in Milan, Italy, we were able to characterize the clinical evolution and conduct additional assays.

The participant was put on treatment with imatinib at 100 mg/day in February 2019, for a myeloproliferative neoplasm with eosinophilia. In January 2022, following unprotected sexual intercourse that occurred in December 2021, he experienced acute retroviral syndrome. He tested positive for HIV (Fiebig stage 5), with exceptionally high viremia at diagnosis ($>10^7$ copies/ml). $CD4^+$ T-cell count at diagnosis was 427/mcl (28%, CD4/CD8 0.46). Genotype resistance testing showed no transmitted drug resistance mutation; subtype was B in all portions. No co-infections and no significant alteration were detected at blood tests (including blood count), and physical examination did not detect any anomaly except diffuse lymph node swelling. cART with TAF/FTC/BIC was started 7 days after diagnosis. Viral load was 10^2 at 1 month from cART start and reached undetectable levels by 6 months, $CD4^+$ count at 6 months was 1172/mcl (56%, CD4/CD8 2). At 24 months from HIV diagnosis, the participant remains healthy on both long-term treatments.

Quantification of total HIV DNA in isolated $CD4^+$ T cells was performed via digital droplet PCR as previously described [11]. We determined 3.7×10^4 HIV-1 DNA copies/10^6 $CD4^+$ T cells before ART initiation and 6.6×10^2 copies/10^6 cells after 1 year of ART, in line with previous studies [12–14]. To investigate the potential of imatinib in impacting HIV infection and, as a consequence, the size of the latent HIV reservoir, we first analyzed SAMHD1 phosphorylation in primary $CD4^+$ T cells isolated from four healthy individuals and treated with two different concentrations of imatinib 1.25 μM and 5 μM, mimicking the 100 mg/day and a standard 400 mg/day drug dose, respectively (Fig. 1a) [15–18].

Western blot analysis revealed that imatinib at 5 μM induced a 5.8-fold reduction in phosphorylated SAMHD1, indicating potential interference with $CD4^+$ permissivity to HIV reverse transcription, whereas imatinib at 1.25 μM induced only a partial reduction that did not reach statistical significance (Fig. 1b). Previous research has highlighted that imatinib was ineffective in blocking SAMHD1 phosphorylation, even at a concentration as high as 10 μM. However, it's important to note that these findings were obtained under conditions of stimulation using anti-CD3/CD8 antibodies or culturing with interleukin (IL)-7 for 5 days, both of which represent stronger stimulation protocols compared to the one we employed, which consisted of only 3 days of IL7-IL15 stimulation [9]. In line with this, when $CD4^+$ T cells were vigorously stimulated with CD3/CD28, mirroring the protocol outlined by Bermejo *et al.*, imatinib did not alter SAMHD1 phosphorylation. However, under milder stimulation with IL7–IL15 for just 3 days, in contrast to CD3/CD28, imatinib exhibited a notable effect even at the lowest concentration tested (1.25 μM), as depicted in Fig. 1c. This suggests that the responsiveness of $CD4^+$ T cells to imatinib in terms of SAMHD1 phosphorylation is contingent upon the strength and nature of the activation stimuli.

We also sought to investigate levels of SAMHD1 phosphorylation *in vivo*, we then performed intracellular staining for P-SAMHD1 in freshly isolated $CD4^+$ T cells from our participant and five other ART-naïve individuals diagnosed with primary HIV infection at similar Fiebig stage, enrolled in the GR-2018-12365699 study and not on imatinib treatment. Interestingly, we observed that three out of five individuals showed a remarkably higher percentage of $CD4^+$ T cells expressing the phosphorylated form of SAMHD1 compared to the participant (Fig. 1d and e).

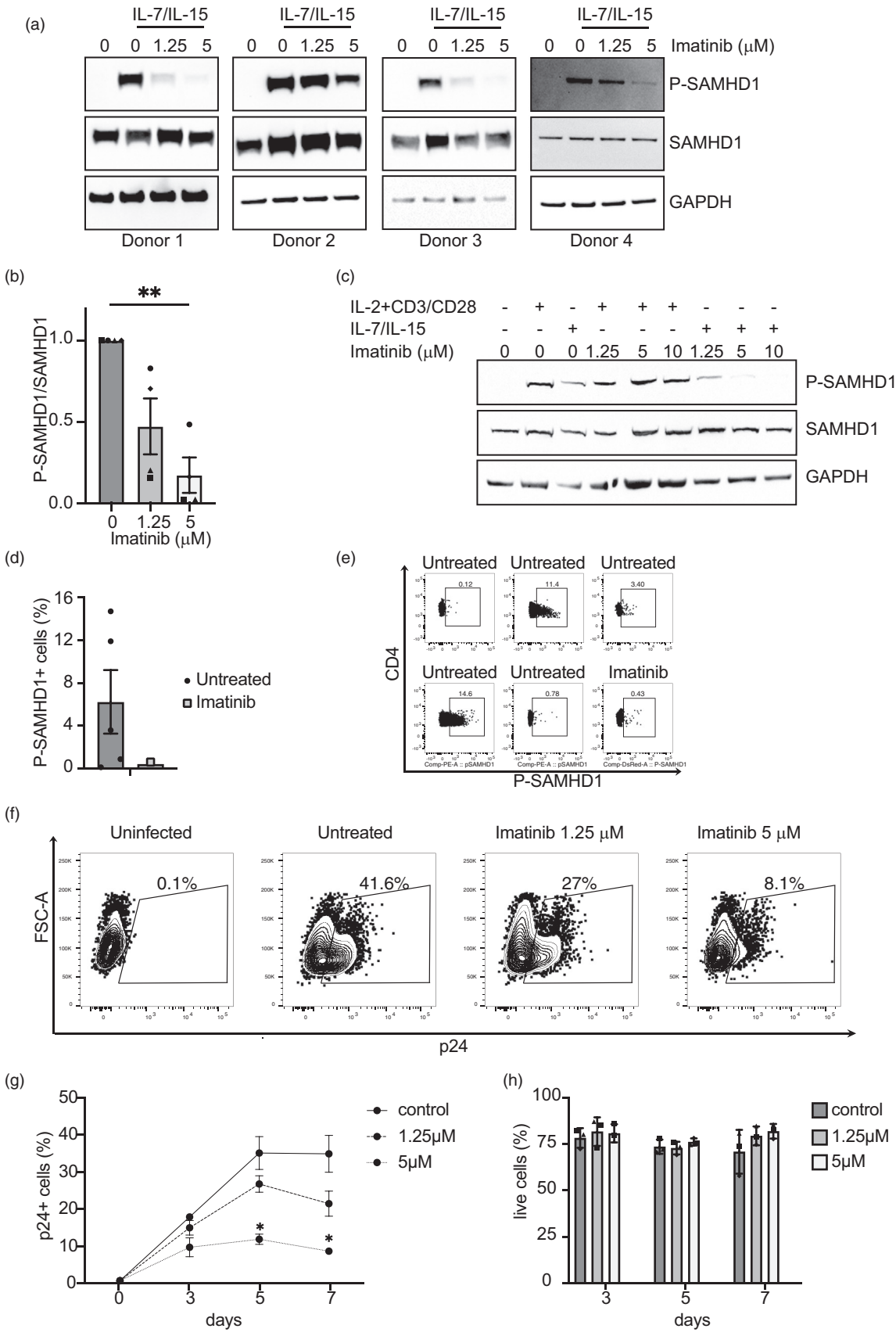

Fig. 1. (a) Western blot analysis of endogenous P-SAMHD1 and total SAMHD1 in primary human CD4$^+$ T cells derived from four distinct healthy individuals, subjected to increasing concentrations of imatinib treatment. (b) Bar graph showing

Fig. 1. Continued

P-SAMHD/SAMHD1 ratio after densitometric quantification. Significance was determined by Friedman test. $^{**}P < 0.01$. (c) Western blot analysis of endogenous P-SAMHD1 and total SAMHD1 in primary human $CD4^+$ T cells stimulated with CD3/CD28, IL7–IL15 or unstimulated as a control and treated with the indicated concentrations of imatinib. (d) Bar graph showing the levels of P-SAMHD1 in $CD4^+$ T cells freshly isolated before ART initiation, from our participant (imatinib) and five other individuals diagnosed at the same Fiebig stage not on treatment with imatinib (untreated). (e) Flow cytometry plots illustrating the gating strategy for P-SAMHD1. (f) Representative flow cytometry plots illustrating HIV infection levels, as measured by p24 expression in primary human $CD4^+$ T cells treated with increasing concentrations of imatinib and infected with HIV-1 pNL4.3 at 7 days postinfection. (g) Graph displaying HIV infection levels in human $CD4^+$ T cells treated with increasing concentrations of imatinib and infected with HIV-1 (averaged across three donors) at 3, 5, and 7 days postinfection. Statistical significance was determined using the Friedman test. * indicates $P < 0.05$. (h) Bar graph showing the viability of $CD4^+$ T cells, determined using viability dye FVS780, BD Biosciences; viability dye, was used to discriminate between live and dead cells treated with increasing concentrations of imatinib and infected with HIV-1 pNL4.3 at 3, 5 and 7 days post-infection.

Finally, we investigated the impact of imatinib on ex-vivo HIV infection of primary $CD4^+$ T cells, isolated from PBMCs from four healthy donors and stimulated with IL7–IL15. $CD4^+$ T cells, isolated from healthy donors and activated with γc-cytokines, were infected with HIV-1 NL4.3. Infection levels were monitored through intracellular staining for HIV p24, followed by flow cytometry analysis (Fig. 1f). We observed a significant suppression at a higher concentration (5 μM). However, imatinib at the 1.25 μM concentration failed to inhibit HIV replication significantly (Fig. 1g). Of note, imatinib had no impact on cellular viability (Fig. 1h).

This case stands out as a distinctive occurrence, marking the first reported instance of acquiring HIV during TKI therapy. The results emphasize the importance of dose considerations, indicating that the participant's 100 mg daily dose might have been insufficient to prevent SAMHD1 phosphorylation and HIV infection in vivo, whereas the higher 400 mg dose might have exerted resistance to HIV.

In summary, the case study explores the potential of imatinib, a TKI, to impact the latent HIV reservoir, presenting a unique scenario of primary HIV infection during TKI therapy. The findings suggest that TKIs may play a role in HIV management, emphasizing the need for further research to understand their potential in HIV treatment and reservoir reduction.

Acknowledgements

The authors thank Valentina Ferroni for invaluable logistic support.

Funding: This study was funded by Italian Ministry of Health RF GR-2018-12365699 study and by Piano di Sostegno alla Ricerca 2022- Linea 2.

Conflicts of interest

There are no conflicts of interest.

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Received: 12 March 2024; revised: 16 May 2024; accepted: 23 May 2024.

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DOI:10.1097/QAD.0000000000003942

Atazanavir-induced lithiasis evidenced several years after drug discontinuation

Atazanavir (ATZ) is a protease inhibitor, used in combination therapy for highly active antiretroviral therapy (HAART) regimen. ATZ may cause stone deposition (lithiasis) at different sites such as urolithiasis [1,2], biliary lithiasis [3,4], sialolithiasis [5], as well as semicircular canals and parotid glands lithiasis [6]. The role of ATZ is not questionable since physical methods, infrared spectroscopy or X-ray diffraction, can demonstrate the presence of the drug or its metabolites within the calculi [1,7]. All cases and series published so far report on the occurrence of lithiasis during ATZ treatment, but we recently observed a case of renal calculi evidenced five years after ATZ discontinuation and revealed by renal colic. To highlight the possible delayed diagnosis of ATZ-induced lithiasis, we here describe this case and provide an analysis of similar cases from the French pharmacovigilance database.

Case report: A 62 years-old woman had been treated with ATZ (boosted with ritonavir) and emtricitabine from August 2005 to October 2018 for HIV infection. Her medical history included hepatitis C, hemochromatosis treated with bloodletting, osteopenia and cannabis abuse. In 2007, ATZ plasma concentrations were high for 2 months, and normalized after ATZ and ritonavir dose reduction. Thereafter, ATZ varied from 0.017 to 0.723 mg/l (normal range 0.2–0.8 mg/l). In October 2007, she also had transient mild hypercalcemia with normal phosphatemia and normal 25-OH-vitamin D3. Vitamin D supplementation was discontinued and hypercalcemia did not reoccur. In May 2008, she was found with asymptomatic microscopic hematuria (10^6 red blood cells/ml), discrete proteinuria (0.3 g/l), negative urine culture and decreased glomerular filtration rate [eGFR (Cockroft) 54 ml/min/1.73 m^2]. In November 2009, she reported right lumbar intermittent pain, but urinary tract ultrasound and abdominal radiography did not evidence kidney lithiasis. Therefore, ATZ was continued under surveillance. In April 2018, because of asymptomatic but persisting decreased kidney function (eGFR 61 ml/min), ATZ was stopped and the treatment was switched to etravirine-raltegravir combination. In March 2023, in the setting of severe urinary tract infection, the patient underwent computed tomography which evidenced right obstructive ureterohydronephrosis due to a 10 mm subpyelic stone, associated with right pyelonephritis outbreaks. The patient received antibiotherapy and JJ stent placement. About 2 months later, cystoscopy under general anesthesia allowed right ureteroscopic fragmentation of the stone. However, extraction was incomplete due to an important ureteral spasm, and not allowing kidney revision. The infrared spectrometric analysis of the ureteral stone fragments revealed a 100% ATZ composition. In September 2023, a 6 mm ureteral stone was still present, and in November 2023, a new extraction was attempted but failed again due to pyeloureteral junction spasm. In March 2024, the JJ stent was removed, but the calculus was considered as not removable, and remained in place.